Behavioral context dependent modulation of descending statocyst pathways during free walking as revealed by optical telemetry in crayfish

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Summary

Crustacean posture control is based on complex interaction of the statocyst input with other sensory inputs as well as the animal's behavioral context. We examined the effects of behavioral condition on the activity of descending statocyst pathways using an optical telemetry system that allowed underwater recording of neuronal signals from freely behaving crayfish. A functionally identified statocyst-driven interneuron that directionally to body tilting without a footboard and to tilting of the footboard was found to show complicated responses depending upon the ongoing behavior of the animal when it freely walked around on the aquarium bottom in the water. The spike firing frequency of the interneuron increased significantly during walking. When the animal stood or walked on the tilted bottom, the interneuron activity could represent the tilt angle and direction if the abdomen was actively flexed, but not if it was extended. Two other statocyst-driven descending interneurons were found to be affected differently by the animal's behavioral condition: the spike activity of one interneuron increased during walking, but its directional response on the tilted bottom was completely absent during abdominal posture movements whereas that of another interneuron was enhanced during abdominal extension only, representing the tilt angle and direction. The results obtained in this study provided the first experimental demonstration that the crustacean postural control under natural conditions is dependent on very fine aspects of the animal's locomotor behavioral context, suggesting far more complex control mechanisms than those expected from the experimental data obtained in isolated and fixed animals.

Introduction

Equilibrium responses for compensating and restoring the original body posture can be elicited independently by sensory signals of different modalities, i.e., equilibrium, somatosensory and visual inputs. The posture control in natural surroundings is ultimately based on complex interaction of these inputs (Horak and Macpherson, 1996; Furudate et al., 1996). Furthermore, it has been shown in many animals including both vertebrates and invertebrates that the activity of central neurons involved in postural control varies significantly depending on the animal's behavioral condition when the sensory stimuli are applied (Deliagina et al., 2000; Takahata and Murayama, 1992). The behavioral context-dependent sensory integration and sensori-motor signal transmission are not specific to postural control but quite general to the control of animal behavior (Delcomyn, 1998; Seki et al., 2003).

The variability in the central neuron responses to sensory stimuli has been studied in general by making physiological recordings from unanesthetized whole-animal preparations performing specific behavior that was restricted to the least extent. In lamprey, for example, extracellular electrodes were chronically implanted to the spinal cord to analyze the descending activity of reticulospinal tract during restricted swimming (Deliagina et al., 2000). Intracellular recording and staining techniques were applied to cricket that were fixed for recording but could walk on a sphere-type treadmill while different kinds of auditory stimuli were present (Schildberger and Hörner, 1988). These studies revealed that the sensory responses of central neurons significantly vary depending on the animal's behavioral context. Having demonstrated in cricket that the gating of sensory responses of higher-order interneurons depended on the animal's walking activity, Staudacher and Schildberger (1998) concluded that significant information about the properties of sensory processing in central neurons can only be gained from experiments in

behaviorally relevant paradigms.

In crayfish, recent studies using intracellular techniques applied to unanesthetized whole-animal preparations have revealed that the control of body and appendage posture depends on not only the complex interaction of statocyst, visual and leg proprioceptor inputs (Okada and Yamaguchi, 1988; Okada et al., 1994; Furudate et al., 1996) but also the behavioral condition of the animal (Murayama and Takahata, 1998a, b; Hama and Takahata, 2003, 2005). Since the recording in the behavioral experiments was made from fixed animals on a belt-driven treadmill or a movable substratum, however, it remains untested how the response characteristics of statocyst interneurons are affected by locomotor behavior in which the postural control becomes most important for the animal.

One approach to the analysis of neuronal activities in freely behaving animals is telemetry, i.e., wireless transmission of neural signals from an animal to the oscilloscope and other recording devices (Fisher et al., 1996; Kudoh et al., 1999; Ando et al., 2002). Whether the recording is made extracellularly from an animal tethered by chronically wired electrodes or intracellularly from an animal fixed on a treadmill to allow mimicked walking, the behavior of such animals has to be restricted to some extent. Valuable information on the neural activity in freely behaving crayfish can certainly be obtained by chronic wired recordings (e.g., Le Ray et al., 2005). There remains, however, a possibility of accidentally or inadvertently hampering the animal's intended behavior. The telemetry system, in particular the transmission device, has its own drawbacks, among which its volume and weight are the most critical since they can potentially hinder intended behavior of the animal. However, under telemetric recording, the animal can perform far wider repertoire of behavior than in the tethered or on-treadmill condition. In the present study, we applied a newly developed optical telemetry system (Tsuchida et al., 2004) to crayfish freely behaving on the tilted bottom of a water-filled aquarium in order to analyze the

activity of descending statocyst-motor pathways in different sensory and behavioral context.

Materials and methods

Animals and preparation

Experiments were carried out on male crayfish, *Procambarus clarkii* (10 - 12 cm in body length, 25-29 g in weight) which were commercially obtained and kept in laboratory tanks. Before operation, each animal was anesthetized in cooled water. A small portion of the dorsal carapace (5 mm x 5 mm) was removed and then the left or right gastric muscle 1 (Katz and Tazaki, 1992) was cut away to reveal the circumesophageal commissures. The activity of statocyst-driven units was recorded from the left or right circumesophageal commissure. The extracellular electrodes made from a pair of Teflon-coated silver wires (125 μm in diameter) and hook-like shaped polyethylene tubes was placed at the area 74 of the circumesophageal commissure (Wiersma, 1958), where the axon of interneuron C_1 was reported to be located (Takahata and Hisada, 1982), and fixed to the dorsal carapace by adhesive. The electrodes were then insulated by Vaseline. To confirm that the spike activity of any statocyst-driven unit was recorded, the animal was manually tilted in the pitch and roll planes. After confirmation of the tilt-dependent spike activity, the removed cuticle was put back on the carapace again and fixed by adhesive. We could confirm that the tip of the recording electrode was staying at or around the original place during experiment by examining the amplitude of the functionally identified unit on the oscilloscope. The experimental animal was rested for more than 1 hour in water. Electromyographic (EMG) recordings were made from the mero-carpopodite flexor muscle of the second walking leg by anatomically placing a pair of Teflon-coated silver wires (125 μm in diameter) on the muscle.

Optical telemetry and video recording

We used a dual-channel transmitter for underwater recording from freely behaving animals. The specifications of transmitter and methodological details have been described

in a previous paper (Tsuchida et al., 2004). Briefly, the amplitude of electrical signals recorded from the animal was modulated to pulse duration using the pulse duration modulation (PDM) method and then the PDM signal was further modulated to intervals of short pulses (2 or 4 μsec duration) using the pulse interval modulation (PIM) method. The PIM signal drove an infrared emission diode to transmit neural information to four underwater receivers that de-modulated to the infrared signals back to neuronal signals (Fig. 1A). De-modulated analog signals, i.e., the spike activity from the circumesophageal commissure and the leg muscle, were stored in a digital audio tape recorder (RD-135T, TEAC; DC-10 kHz) for later analyses. In some experiments, the recording was made in the air. The telemeter system could reliably transmit signals in those experiments.

Crayfish mounted with a transmitter and electrodes could freely walk in the experimental aquarium (40 cm width x 60 cm depth x 40 cm height) filled with water to a depth of 15 cm (Fig. 1A). The floor of the experimental aquarium was tilted by 10°. When the animal walked on it, the body was tilted depending on the orientation of the animal body in relation to the tilt direction of the floor. PIN (p-intrinsic-n) -photodiode receivers were placed at each corner of the square (30 cm x 30 cm) shown in Fig. 1A. In this condition, stable recording could be obtained in the dark-blue square region (40 cm x 40 cm). The behavior of crayfish was simultaneously video-recorded from above using a digital video camera (DCR-TRV9, SONY; 30 frames/sec). The area covered by the camera image was 40 cm x 60 cm (Fig. 1B). Experiments were carried out in the dark, but the position and head direction of the animal were clearly determined. Video recording of the animal behavior from above was always done with periodically flashing, short-duration (100 msec) light signals (green LED) at 0.5 Hz. The electrical signals for controlling these light flashes were recorded together with the neural and muscle signals for synchronizing video data with electrophysiological ones.

Validity of telemetric recording

We reported that the newly developed optical telemetry system can reliably transmit neural signals within a rectangle (30 cm x 40 cm) in the freshwater (Tsuchida et al., 2004). The frequency range was narrower than in wired transmission so that small-sized spikes could be hardly discriminated in the wirelessly transmitted record due to its low signal/noise ratio. The system, however, had a wide-enough frequency range to transmit medium- and large-sized neuronal spikes recorded extracellularly in the present study. Hence the problem we had to consider in applying the telemetry system to the current experiment was whether or not the transmitter and the electrode mounted on the animal (Fig. 1C, D) hampered its normal behavior. We video-recorded the free walking behavior of experimental animals with the transmitter mounted and unmounted on their back. Although we did not quantitatively analyzed the walking behavior in this study, qualitative comparison of the walking behavior in the two conditions indicated that the mounted transmitter did not affect the animal behavior in any serious way.

Data processing

Physiological data were digitized at 40 kHz using an A/D converter (PowerLab, ADInstruments) and software (Chart v4.2, ADInstruments). All spikes were sorted depending on its amplitude and duration at the half amplitude (Schmidt, 1984) using another software (Spike Histogram Extension for Chart v4.2, ADInstruments). All spikes automatically sorted were manually re-sorted using our homemade softoware depending on their form. The position and head direction of the animal were plotted at the rate of 15 frames/sec (0.5 sec interval; Fig. 1B).

Results

We made successful unit recordings from more than 40 animals. Sixteen animals showed directional responses to body tilting in the air without a leg substratum, but only four of them showed sustained responses to body tilting during walking in the aquarium. Other animals responded just transiently to body tilting whereas another some showed variable responses in the same behavioral context and tilted situation. Since our aim was to analyze the neural activity involved in the central mechanism of behavioral context-dependent posture control, we selected for the present study those four animals that reliably showed directional responses of statocyst-driven units during free walking. In one of them, the statocyst-driven unit became unresponsive during experiment in the aquarium. We continued experiment, however, to analyze the activity characteristics of non-statocyst units. The data obtained from the statocyst unit of this animal in the early experimental stages were excluded from the current analyses.

Behavioral context dependent unit activities

Twenty-one units in all, obtained from the selected three animals, were found to change their spike activity when they were engaged in locomotor behavior (Table 1). Four unit activities extracted from multi-unit recordings are illustrated in Fig. 2A. In two of them, named unit 3c and 3d, the activity during forward walking was different from that during backward walking (Fig. 2B): the spike discharge rate of the unit 3c was 3.1 ± 0.6 spikes/sec (mean ± SE) during forward walking while it was significantly higher (11.7 ± 0.4 spikes/sec; $p < 0.01$ in the two-sided Mann-Whitney U-test). The unit 3d showed a similar tendency: the spike discharge rate was lower during forward walking (14.5 ± 1.2 spikes/sec) than during backward walking (18.7 ± 0.5 spikes/sec). The units 3a and 3b, in contrast, showed no significant change in their spike activity whether the animal walked

forwardly or backwardly (Fig. 2B).

All four statocyst-driven units were found to change their spike activity depending on the animal's behavioral condition (Table 1). Their response characteristics are described in the following sections. Units 1d and 2c were judged to be the same unit on the basis of their responsiveness to the recorded-side-down and head-up tilting, their activity dependence on specific behavioral conditions and their relative amplitude in the multi-unit recording from the circumesophageal commissure.

Activity of an identifiable descending unit during body tilting

When the spike activity of the ventral nerve cord is recorded at the circumesophageal commissure, a medium-sized spike that is smaller in amplitude than the semi-giant spikes (Wilkens and Larimer, 1973) but characteristically larger than many other small units is unambiguously recognized. This unit was designated interneuron C_1 and its directional responses to body tilting have been studied in detail under restricted conditions: it is characterized by tonic excitatory responses to head-up and recorded-side-down tilting in the air (Takahata and Hisada, 1982, 1985). In this study, we first analyzed the response of this unit when the animal was engaged in locomotor behavior in unrestricted conditions. It showed a low level of spontaneous spike discharge in the upright body position when the animal was at rest. As reported earlier, the interneuron showed directional responses to body tilting in the air without a footboard or substratum (Fig. $3A_1$). Thus, ipsilateral-side-down tilting (90°) increased the spike activity to 2.8 ± 0.7 spikes/sec, but the activity of the interneuron C_1 was completely absent during contralateral-side-down tilting (Fig. $3A_1$). Since only the statocyst was activated in this situation, the interneuron responses to body tilting were driven solely by the statocyst input.

Directional responses were also observed in the interneuron when the animal stood or

walked on the substratum tilted by 10° (Fig. 3B1). Compared with the firing rate observed when the animal was tilted in the air, the interneuron showed a higher firing rate (8.6 ± 0.9 spikes/sec) when the animal was on the substratum tilted in the same direction as in the body tilting in the air (Fig. 3B2). Although the tilted angle of the substratum (10°) was smaller than that of the animal body in Fig. 3B1, the firing rate was much greater. It should be noted here that the experimental animal was not fixed to the upright body position nor to the substratum in this study. The animal therefore showed equilibrium reflexes of legs on the tilted substratum so that the animal body was less tilted than the substratum. Since the animal successively changed its posture on the substratum, we could not measure the exact angle of body tilt on the substratum. The interneuron thus received complex combination of statocyst and leg proprioceptor inputs during standing and walking on the tilted substratum. The larger response of the interneuron to substratum tilting than to body tilting suggested that the two modalities of sensory inputs would make facilitatory summation.

Activity of interneuron C1 during resting and walking

We previously showed that the spontaneous spike activity and the sensory responses of many statocyst-driven descending interneurons were significantly modulated by free leg movements in the air, receiving central inputs from the locomotor center (Hama and Takahata, 2003). The interneuron C1, however, was not affected by leg movements in the air (Fig. 4). When the animal was held upright in the air, the interneuron showed spontaneous spike discharges (0.4 ± 0.4 spikes/sec; Figs. 3A1 and 4B1). In response to body tilting in the ipsilateral-side-down direction, the interneuron increased its spike activity to 1.5 ± 0.4 spikes/sec (Fig. 4A1). When the animal was engaged in free leg movements in the air, an increase was observed in the muscle activity (upper trace in Fig.

4A2) and in the spike activity of the circumesophageal commissure, but neither the spontaneous spike activity nor the directional response was affected (bottom trace in Fig. 4A2). The animal body was kept tilted during the whole period of recording shown in Fig. 4A1 and A2. In one exceptional case, the interneuron showed an increase in the spontaneous spike discharge during active leg movements while the animal body was kept upright (Fig. 4B2). We examined the interneuron C1 activities associated with leg movements 39 times in 2 animals and this exceptional activity was observed in only 1 case. Visual observation and EMG recording could not discriminate any difference in the behavioral condition between those two cases shown in Figs. 4A2 and 4B2. It therefore remained unknown what behavioral parameter affected the interneuron activity during active leg movements in the upright body position. Our observation suggested that the interneuron activity was mostly invariable, independent of the animal's behavioral state, when the legs were maintained in the air without a substratum. Thus, the interneuron activity was not affected at all when the animal extended its abdomen endogenously in the air (downward arrows in Fig. 4C).

The leg and abdominal movements in the air and water off the bottom are seldom observed in the locomotion of crayfish. In order to analyze the interneuron activity in the context of ongoing locomotor behavior under natural conditions, we applied the optical telemetry techniques to freely walking animal in the water. The spike activity of the interneuron C1 at the onset of and during endogenously initiated walking is illustrated in Fig. 5. When the animal was at rest on the substratum without sideward tilting, i.e. longitudinal axis of body directed to 0° or ±180°, few spike activities were observed in the circumesophageal commissure. The firing rate of the interneuron was also low (2.8 ± 0.3 spikes/sec; Fig. 5A). When the crayfish endogenously initiated walking on the substratum without sideward tilting (dashed line in Fig. 5A), the spike activity of the

circumesophageal commissure became high (top trace in Fig. 5A). This high level of spike activity was contributed by increases in both ascending and descending spike discharges transmitting sensory and motor command signals respectively. The interneuron C_1, which is one of the descending interneurons, also increased its spike activity and maintained the high discharge frequency during walking (bottom traces in Fig. 5A and 5B). Statistical analyses revealed that the interneuron showed a higher rate of spike discharge during walking on the substratum without sideward tilting than at rest ($P < 0.05$, two-sided Mann-Whitney U-test). The spike activity of the interneuron during walking was not invariable, however, showing a certain degree of fluctuation since walking behavior activated internal and external mechanoreceptors of walking legs in a complex way.

Using intracellular recording and staining techniques, we previously showed that some statocyst-driven descending interneurons enhanced their responsiveness to statocyst input when the animal actively moved walking legs on a substratum (Hama and Takahata, 2003). Since we could not identify the interneuron C_1, it remained unknown how the interneuron activity was affected during walking. In the present study, the extracellular recordings from freely behaving animals revealed that the spike firing frequency of the interneuron was 6.6 ± 0.7 spikes/sec when the animal walked on the substratum tiled in the contralateral-side-down direction while it was significantly greater (8.5 ± 0.4 spikes/sec) when the animal walked on the substratum tilted ipsilaterally ($P < 0.05$, two-sided Mann-Whitney U-test). More detailed analyses revealed that the interneuron retained directional responsiveness over the full range of body axis orientation (Fig. 5C). The recording electrode was placed on the left circumesophageal commissure in this experiment. When the longitudinal axis of the animal body was perpendicular to the tilt direction of the substratum, the side of commissural recording was either lowered (-90^o) or lifted (90^o) (see Materials and methods). There was no sideward tilting when the animal

body was oriented in 0º and ±180º directions. The animal took a variety of intermediate orientation angles during free walking. The spike activity of the interneuron C_1 tended to increase when the animal walked on the substratum tilted in the ipsilateral-side-down direction and decrease in the contralateral-side-down direction. However, no significant difference was observed between any tilt angles ($P > 0.05$, ANOVA).

Effects of abdominal posture on the interneuron activity

The equilibrium responses of crayfish are significantly modulated by behavioral context. Uropod steering in response to body rolling (Yoshino et al., 1980), for instance, is enhanced by abdominal extension during locomotion and suppressed during turning behavior (Takahata et al., 1984). However, as shown in Fig. 4C, the abdominal posture movement in the air itself did not affect the spike activity of the interneuron C_1. We thought that the abdominal posture movement evoked in the air should differ in the leg proprioceptor activation from that evoked during locomotion on a substratum, and therefore analyzed the interneuron activity changes associated with the abdominal movements during locomotion.

A typical record of the interneuron C_1 activity during free walking on the tilted substratum is illustrated in Fig. 6A. The animal was at rest or pausing with its head up, the substratum being bilaterally symmetrical for the animal for 26 seconds after the recording was started (P_1 in Fig. 6B). It then showed forward walking, gradually changing the head direction rightward with the abdomen extended (Ab. Ex. FW). During this forward walking (27 - 64 seconds), the animal showed an asymmetrical posture of bilateral uropods, i.e., the exopodite on the lifted side was closed whereas that on the lowered side opened. After a pausing period (65 - 97 seconds; P_2), the animal began backward walking, gradually changing the head direction rightward with its abdomen flexed (solid line in Fig.

6B; Ab. Fl. BW). The spike activity of the interneuron C_1 was higher during backward and forward walking than at rest. The interneuron activity was much higher during abdominal flexion than during extension. When the animal was just standing and not walking, no significant change was observed in the interneuron activity between abdominal flexion and extension. During abdominal extension in walking, the general activity of the interneuron and its directional responsiveness to tilting were both significantly lower than those during abdominal flexion (Fig. 6C). In this experiment, the interneuron activity was recorded from the circumesophageal commissure on the left side. It increased when the animal walked on the substratum tilted in the directions of 0º - -135º and 0º - 45º, i.e., in the ipsilateral-side-down or head-up directions. On the other hand, the interneuron activity decreased when the animal walked on the substratum tilted in the contralateral-side-down or head-down directions (45º - 180º and -135º - -180º). The interneuron activity became largest when the animal body was oriented in the directions of -90º - -135º (26.4 ± 5.5 spikes/sec) and 0º - -45º (24.8 ± 3.6 spikes/sec). When the animal was engaged in abdominal extension during walking, the interneuron activity showed a drastic change: the general activity became significantly lower than that recorded during abdominal flexion and the maximal response to substratum tilting was observed in the directions of 135º - 185º and 0º - -45º. As described above, the body orientation in 0º - -45º direction corresponds to head-up tilting of the animal body while the orientation in 135º - 180º direction to head-down tilting.

Activities of other statocyst-driven descending interneurons

The abdominal extension that enhances the uropod steering behavior in response to body rolling was found to suppress the response of the interneuron C_1 to body tilting. We looked for other statocyst-driven descending interneurons that would increase their

responsiveness to body tilting during abdominal extension in walking. Since it depended on the electrode positioning relative to the commissure what kind of units could be recorded, we intentionally changed the positioning angle and depth of the electrode to obtain other units than the interneuron C_1. In the experiment illustrated in Fig. 7, two descending units were found to respond directionally to body tilting. One of them (unit A) increased its firing frequency when the animal body was tilted in the ipsilateral-side-down direction whereas another one (unit B) was activated when tilted in the contralateral-side-down direction (Fig. 7A). Thus the response directionality of the unit A in body rolling was similar to that of the interneuron C_1, but head-up and -down tilting elicited no directional response in units A and B (data not shown). We analyzed the effects of abdominal posture movements upon the responsiveness of these two units to the body tilt during walking. In the experiment illustrated in Fig. 7, the recording was made from the left circumesophageal commissure. When the animal body was oriented in the direction of -45º - -135º, the substratum was tilted in the ipsilateral-side-down direction. The orientation in the direction of 45º - 135º indicated that the animal walked on the substratum tilted in the ipsilateral-side-up direction. The unit A clearly responded to body tilting when the animal was at rest (Fig. 7A). This directional response, however, was not observed during walking with the abdomen either flexed or extended actively (left panel in Fig. 7B).

The unit B, on the other hand, showed different responses from those of the unit A and interneuron C_1. During walking with the abdomen extended, the spike activity became maximal when the animal body was oriented in the direction 90º - 135º. It then decreased as the orientation angle changed to -45º to -135º (right panel in Fig. 7B). The directional response of the unit B during walking with the extended abdomen was larger than that observed at rest. In contrast, the general activity was enhanced, but the directional response to body tilting was not observed in the unit B when the animal walked with its abdomen

flexed. These findings suggested that the facilitation of uropod steering response during walking with abdominal extension is subserved by other descending interneurons than the interneuron C_1 such as the unit B.

Discussion

The responsiveness of central neurons to sensory stimuli varies dramatically depending on ongoing behavior of the experimental animal (e.g., Schildberger and Hörner, 1988; Staudacher and Schildberger, 1998; Staudacher, 2001). The behavioral context-dependent modulation of sensori-motor pathways becomes particularly critical in postural control since it is during locomotion that the postural control mechanism is most needed. In the present study, we applied our optical telemetry technique (Tsuchida et al., 2004) to freely behaving crayfish and recorded statocyst-related central neuron activity in order to analyze how the descending statocyst-motor pathway is modulated during locomotion under natural conditions. The results suggested that the crustacean posture control is based on neuronal mechanisms far more complex than those expected from the experimental data obtained in animals fixed in the air (Takahata and Hisada, 1985) or on a treadmill apparatus (Murayama and Takahata, 1996).

Behavioral context dependent unit activities in the circumesophageal commissure

The current method of optical telemetry revealed that many units descending from the brain to the thoracic and abdominal ganglia changed their spike activity depending on the behavioral condition of the animal (Fig. 2). Since we confined detailed analyses to the statocyst descending system in this study, our description on other units inevitably remained fragmentary and episodic (Table 1). Nevertheless, we could find several interesting aspects in the behavior-dependent control of unit activities descending from the brain for the first time by applying newly developed telemetry techniques to freely behaving animal. First, some units were reliably increased their spike discharge rate when the animal engaged in specific behavior. Their activity even preceded the initiation of limb movements (unit 3c in Fig. 2B). These descending unit activities presumably represent the

motor command (Edwards et al., 1999; Esch et al., 2002) for initiating or maintaining specific locomotor movements, and we are currently analyzing these activities by intracellular as well as extracellular techniques. Second, not all descending units changed their spike activities depending on the animal's behavioral condition. We could not identify those units that showed invariable spike activities all the time, but some of them are thought to be descending sensory interneurons since we previously reported those interneurons that kept constant responsiveness regardless of the animal's behavioral and sensory conditions (Hama and Takahata, 2003). Finally, some descending units showed great variability in not only their spontaneous spike discharge but also their responsiveness to specific sensory stimuli depending on the animal's behavioral condition as discussed in the following sections.

It is interesting to note here that both the activation of motor-related units (3c and 3d in Fig. 2) and the modulation of sensory responsiveness in statocyst-driven units (Figs. 3-7) are not associated with general activity changes but evoked behavior-specifically. This finding confirms the classical hypothesis (Sperry, 1950; von Holst and Mittelstaedt, 1950) that the motor center responsible for specific behavior generates not only motor commands for initiating and controlling the behavior but also efference copy signals to prepare the animal's sensory and motor systems for the behavior as in the saccadic suppression of vertebrates (Thiele et al., 2002) and the escape behavior of crayfish (Wine and Mistick, 1977) . It remains to be studied further whether the motor-related activities of the units 3c and 3d in Fig. 2 reflect the motor command signals directed toward lower-level motor circuits or the efference copy signals to be distributed in the nervous system. The modulation of sensory responsiveness in statocyst-driven units during locomotor behavior discussed below (Figs. 3-7), in contrast, is thought to be primarily based on the efference copy signals originated from the locomotor center in the brain.

Modulation of descending statocyst pathways by sensory and behavioral conditions

Descending statocyst pathways were found first to involve four interneurons each showing different directional sensitivities (Takahata and Hisada, 1982) and later to involve 14 other interneurons in the circumesophageal commissure (Hama and Takahata, 2003). Using intracellular recording techniques applied to fixed animals, we demonstrated that the synaptic responses of each descending statocyst interneuron to statocyst stimulation were differently modulated in the brain by leg movements in different conditions (Hama and Takahata, 2003). Thus some interneurons showed enhanced responses due to synaptic summation of inputs from the statocyst and leg movement system regardless of whether a substrate was provided or not, whereas others showed more effective summation when a substrate was provided during leg movements than when it was not. In one interneuron, the synaptic response to statocyst stimulation was never affected by leg movements either on a substrate or in the air. Of these three groups, our present finding suggested that the interneuron C_1 belongs to the second one, but there was a little difference between the previous interneurons and the interneuron C_1 in that the C_1 activity was not only enhanced but suppressed depending on the behavioral condition of the animal (Fig. 6). Response modification by sensory and behavioral conditions has been extensively reported in many sensori-motor systems of invertebrates (Staudacher, 2001; Frost et al., 2003) and vertebrates (Deliagina et al., 2000; Seki et al., 2003). In most cases, however, the activity of single neurons is either enhanced or suppressed. The interneuron C_1 therefore shows quite a novel type of response variability that is unprecedentedly complex.

The spike responses of the interneuron C_1 to body rolling was not affected at all by active leg movements as well as abdominal posture movements in the air (Fig. 4) confirming the previous result obtained by conventional extracellular recordings from

fixed animals (Takahata and Hisada, 1985). When the animal walked in the water on the horizontal aquarium bottom that was bilaterally horizontal, the spontaneous activity was significantly greater than at rest (Fig. 4B). The interneuron C_1 activity was thus affected differently by leg movements in the air and on a substratum. It is difficult at this time to infer any difference in the central nervous activity between during leg movements in the air or water and during walking on a substratum since no experimental data is available in this regard. The situation, however, can be reduced to the leg movements with and without a specific load or disturbance. Although there are a variety of mechanoreceptors including position detectors (Mill, 1976) and stress detectors (Marchand et al., 1995) in the walking legs of crayfish, the nerve signals from these mechanoreceptors to the brain are filtered by centrally generated signals through presynaptic inhibition during locomotion (Cattaert et al., 1990, 1992; El Manira et al., 1991). This situation implies that the sensory signals themselves can remain constant in different load condition. It is therefore suggested that the different effects on the interneuron activity of leg movements in different conditions are due, at least partly, to a difference in this centrally programmed peripheral filtering or cancellation. The possibility still remains, needless to say, that different leg sensory signals due to different load conditions directly affect the interneuron activity.

The interneuron C_1 activity was also affected differently by abdominal posture movements even when the animal was engaged in the same walking on the aquarium bottom (Fig. 6). Other interneurons were also found to be affected by abdominal movements but in different ways from the interneuron C_1 (Fig. 7). Since the crustacean abdomen is equipped with muscle receptor organs (MROs) that monitor the stretch of abdominal extensor muscles (Wiersma et al., 1953), the different effects of abdominal movements on the interneuron activity can be due to either central signals that command the abdominal movements (Larimer and Moore, 2003) or peripheral signals from the

MROs. Further study is needed to clarify to what extent each signal contributes to enhance or suppress the descending interneuron activity during abdominal movements and walking.

Neuronal mechanisms underlying behavioral-context dependent posture control

In some sensori-motor systems, the pathway transmitting specific sensory information to the motor system operates independently of the animal's behavioral condition. For example, in the locust flight control system, the sensory signal from ocelli for steering control is transmitted to premotor interneurons invariably regardless of whether the animal is engaged in flight behavior or not (Reichert and Rowell, 1985). The steering control of the flight behavior is based on synaptic summation of the ocelli input in the premotor interneurons with the central input from the flight pattern generator so that the steering posture is taken at a certain phase of the rhythmical flight behavior (Reichert and Rowell, 1986). Similar reflex gating (Delcomyn, 1998) has been reported in many other sensori-motor systems (Staudacher, 2001; Frost et al., 2003). Gating of sensory information by other sensory inputs or by behavioral context is also common in the vertebrate brain (Deliagina et al., 2000; Seki et al., 2003).

In the uropod steering of crayfish with walking legs off the substratum, it has been shown that the statocyst pathways descending from the brain are not only gated by abdominal posture movements in the terminal abdominal ganglion to activate the uropod motor system; they also activate in anterior abdominal ganglia other descending pathways that originate there and run in parallel with the original pathways to converge onto the uropod motor system in the terminal ganglion, thus constituting a multiple gate control system (Takahata and Murayama, 1992; Fraser and Takahata, 2002). The statocyst sensory signal is multiplied by a cascade of abdominal interneurons to enhance the synaptic response of the uropod motor system (Murayama and Takahata, 1998a, b). The present

study using optical telemetry techniques applied to freely behaving animals in the water clarified another aspect of the crustacean postural control system: the behavioral context dependent changes in the statocyst sensory signal pathway. Responses of a functionally identified descending statocyst interneuron to body tilting were affected in different ways by leg movements and abdominal posture movements depending on the sensory and behavioral conditions (Figs. 5, 6). We also showed that different descending interneurons were affected differently by the same sensory or behavioral condition (Fig. 7).

The present findings do not negate the importance of multiple gate control in the postural control of crayfish since the spike activities of descending statocyst interneurons can elicit only subthreshold synaptic potentials in the uropod motor neurons (Takahata, 1990). However modulated by behavioral context, therefore, they will not be capable by themselves of eliciting spike activities in motor neurons. They have to make synaptic summation with central inputs from the locomotor system (Murayama and Takahata, 1998a). Although our previous study demonstrated that the sensory signal carried by the interneuron C_1 and gated to the uropod motor neurons was invariable in the fixed animal with legs off the substratum (Takahata and Hisada, 1985), it was not the case in the freely behaving animal. In a particular behavioral context, i.e., during abdominal extension, the interneuron C_1 activity does not represent the angle of body tilt that is reliably represented by the same interneuron during abdominal flexion (Fig. 6). The responses of other interneurons to tilting were affected differently from those of the interneuron C_1 (Fig. 7). The posture of the animal body during walking is thus controlled by different interneurons, the combination of which is changing every moment depending on the behavioral context of the animal. The reflex gating of sensori-motor pathways is surely one aspect of postural control in the freely behaving animal, but the gating is organized in multiple ways and, furthermore, complex mechanisms modulate the sensory information before the gating

mechanism converts it into motor output signals. Do the interneurons that are affected differently by sensory and behavioral conditions all converge onto the uropod motor system so that their signals are non-selectively gated to the uropod motor system? Alternatively, is the sensory information that is relevant at the moment selected by another upstream mechanism to be gated by central signals from the locomotor system to activate the motor system? Further study is needed to clarify these questions.

Figure legends

Figure 1 Experimental set-up. **A:** Arrangement of the telemetry and video-recording systems. Crayfish was placed in an experimental aquarium filled with water to a depth of 15 cm. The bottom floor was tilted by 10˚. Four PIN-photodiodes (PIN PDs) as receivers were placed at the corners of a square of 30 cm x 30 cm. An LED was driven by synchronous signals that were simultaneously fed to a DAT recorder together with nerve and EMG signals. Due to the optical signal decay along the distance from the transmitter, the recording was reliable only in a limited area. The area illustrated in dark blue indicates that the telemetric transmission was secure there. The pale-blue indicates water in the aquarium. **B:** The angular coordinate adopted in the present study to describe the direction of the animal body orientation. The head direction and behavior of crayfish were video-recorded from above. **C:** The optical transmitter mounted on the animal. Wire electrodes for EMG recording from the mero-carpopodite flexor muscle and the chronic electrode for extracellular recording from the circumesophageal commissure were connected to the dual-channel transmitter. **D:** Schematic drawing of the chronic electrode for extracellular recording from the circumesophageal commissure. Wire connection from the electrode to the transmitter is shown by broken lines. The commissure was hooked up lightly by the electrode to secure the recording during free movements. Two dashed ovals are lithium cells for the transmitter. They were omitted from the illustration in C for the sake of clarity. IRLED; infrared LED.

Figure 2 Unit activities sorted out by discriminating software from extracellular recordings of the whole circumesophageal commissure. **A:** Raw recording data during walking (upper trace) and its partial expansion (lower trace). Four units could be discriminated in this example as indicated by four different symbols. **B:** Activity of each unit during forward

walking (left panel) and backward walking (right panel). The histograms were based on 1 sec time bins. The units 3a and 3b showed almost the same activity during forward and backward walking. The units 3c and 3d showed higher activities during backward walking than during forward walking. The vertical dashed lines indicate the time of walking behavior onset. The thick bar at the bottom indicates the expanded part of the data shown in **A**. The insets on the right show superimposition of each unit. They are expanded in the time scale as indicated but are the same size in the voltage scale as in **A** except the unit 3a that was reduced to the half of **A** in the voltage scale.

Figure 3 Responses of the interneuron C_1 to body and substratum tilting. **A_1:** Body tilting by 90˚ in the air. Top trace shows the spike activity recorded extracellularly from the circumesophageal commissure (CC). The spike activity of the interneuron C_1 was isolated electronically from the commissural recording and are shown in the second trace (C_1). The bottom trace monitors the animal body tilting, the upward and downward deflection indicating ipsilateral-side-down (ISD) and contralateral-side-down (CSD) tilting in which the side of commissural recording was lowered and lifted respectively. **A_2:** Statistical comparison of the interneuron activities between horizontal, ipsilaterally tilted and contralaterally tilted positions. The inset shows superimposition of electronically isolated spikes in A_1. **B_1:** Substratum tilting by 10˚. The animal was standing on the tilted substratum. Since the animal was free to evoke postural reflexes, the exact angle of body tilt on the tilted substratum was unknown. The recording for each positioning was made intermittently whenever the freely behaving animal met the behavioral and orientation requirements. **B_2:** Statistical comparison of the interneuron activities.

Figure 4 Effects of leg and abdominal movements on the interneuron C_1 activity. **A:** Body

tilting in the contralateral-side-down direction without a leg substratum when the animal was at rest (**A1**) and actively moving legs (**A2**). The animal body was kept tilted during the recording shown in A1 and A2. The top trace monitors the muscle activity whereas the bottom one the interneuron C1 activity. **B:** An exceptional case in which the interneuron activity was affected by leg movements in the air. The spike activity during maintained tilt of the resting animal (**B1**) was significantly enhanced when the animal actively moved walking legs (**B2**). **C:** Effects of abdominal posture movements on the interneuron activity. Upward and downward arrows indicate the onset time of abdominal extension and flexion movements respectively. No noticeable change was observed in the interneuron activity during these movements.

Figure 5 Interneuron C1 activity during free walking. **A:** Recordings from an animal that spontaneously initiated walking (vertical dashed line) on the horizontal bottom of the aquarium. Top trace shows extracellular recording from the circumesophageal commissure (CC), second trace EMG recording from the mero-carpopodite flexor muscle of the right second leg, third trace the interneuron C1 activity that is also represented in the form of a frequency histogram with the time bin of 1 sec at the bottom. The inset shows superimposition of interneuron spikes discriminated from the commissural recording. For clarity, the superimposed record is enlarged in both the time and voltage scale. The former scale is provided in the figure while the latter factor was 3.0 relative to the raw data. **B:** Statistical comparison of the interneuron C1 spike activity between the resting and walking conditions. **C:** The interneuron activity when the animal walked on the tilted bottom in different directions. The angular coordinate is shown in Fig. 1B. When the animal walked in 0˚ and 180˚ directions, the bottom was bilaterally symmetrical for the animal body. In the direction of 90˚ and -90˚, the animal body was tilted in the contralateral-side-down and

ipsilateral-side-down directions respectively. Shaded bar graphs were obtained in the condition that the animal walked on the substratum tilted in the contralateral-side-down directions.

Figure 6 Interneuron C_1 spike activity dependent on the abdominal posture movements during free walking. **A:** Tracing of two bouts of walking on the tilted bottom. The body position and head orientation are plotted at 1 sec interval. The filled circle indicates the head while the straight line the longitudinal body axis of the animal. In the first bout of walking, the animal walked forwardly with the abdomen extended (Ab. Ex. FW). The animal then turned left and started the second walking in the backward direction with the abdomen flexed (Ab. Fl. BW). Smooth-line arrows in the figure indicate approximate displacement of the animal. **B:** Spike activity of the interneuron C_1 during the walking shown in A. Represented in the form of a frequency histogram with the time bin of 1 sec, the record shows that the interneuron activity was enhanced when the animal started abdominal flexion (indicated by a vertical line). The crayfish behavior is shown at the top: P, pause; Ab. Ex. FW, forward walk with abdomen extended; Ab. Fl. BW, backward walk with abdomen flexed. The head orientation is monitored by the second trace. **C:** Spike activities of the interneuron C_1 in different orientation angles. Filled and open bars indicate the spike activity during abdominal flexion and extension respectively.

Figure 7 Effects of abdominal posture on descending interneurons other than the interneuron C_1. **A:** Two statocyst-driven descending units in the circumesophageal commissure. The original recording is shown at the top (CC) and two unit activities (A and B) discriminated from the record are shown in the second and third traces. The inset on the right show superimposition of isolated two units. For clarity, the superimposed records are

enlarged in both the time and voltage scale. The former scale is provided in the figure while the latter factor was 5.0 relative to the raw data for both units. The bottom trace monitors the body tilt angle. The unit A responded directionally to ipsilateral-side-down tilting (ISD) whereas the unit B to contralateral-side-down tilting (CSD). **B:** Spike activities during walking in different orientation angles with the abdomen extended (open bars) and flexed (filled bars). The unit A (left) showed no directional responses when the animal extended or flexed its abdomen whereas the unit B (right) could represent directional information when the animal extended its abdomen. The unit B showed no directional responses during abdominal flexion.

Table 1 Activities of all units obtained from the three animals that showed reliable responses to body tilting and another animal that showed reliable responses in the early stage of experiments but became later unreliable, yet remained in the recording condition for analysis of non-statocyst units. The second column from the left shows the responses of each unit to body tilting. The third and fourth full columns summarize the activities during resting and walking respectively. In the third column, the subcolumns compare the activity between abdominal extension and flexion and between resting and walking. In the fourth column, the subcolumns compare the activity between forward and backward walking and between abdominal extension and flexion. The symbols ">>" and "<<" mean that the unit activity was significantly different in respective direction ($p < 0.05$; Mann-Whitney's two-sided U-test). The units 1d and 2c corresponded the interneuron C_1 whereas the units 4a and b were designated units A and B respectively in the text.

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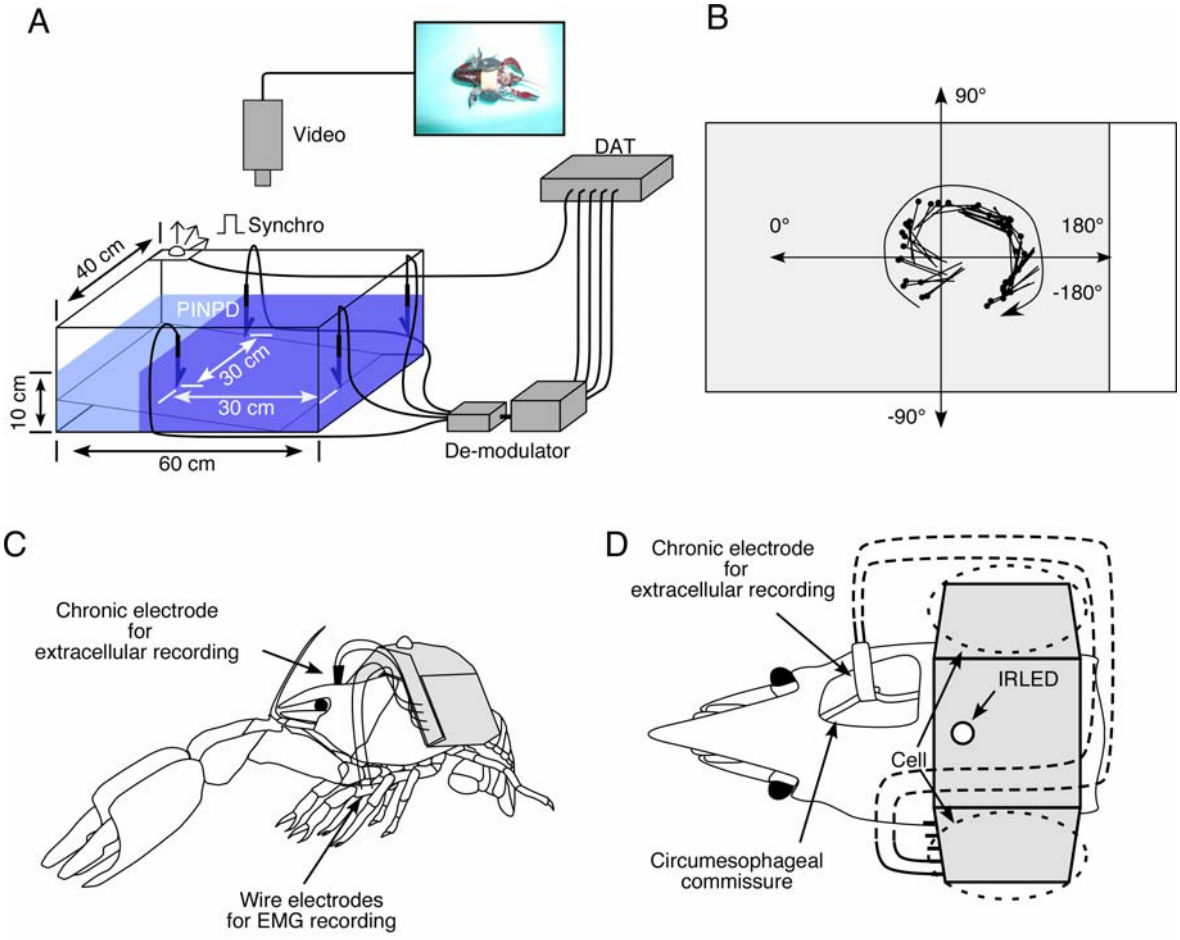
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DAT
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-90°
C
Chronic electrode
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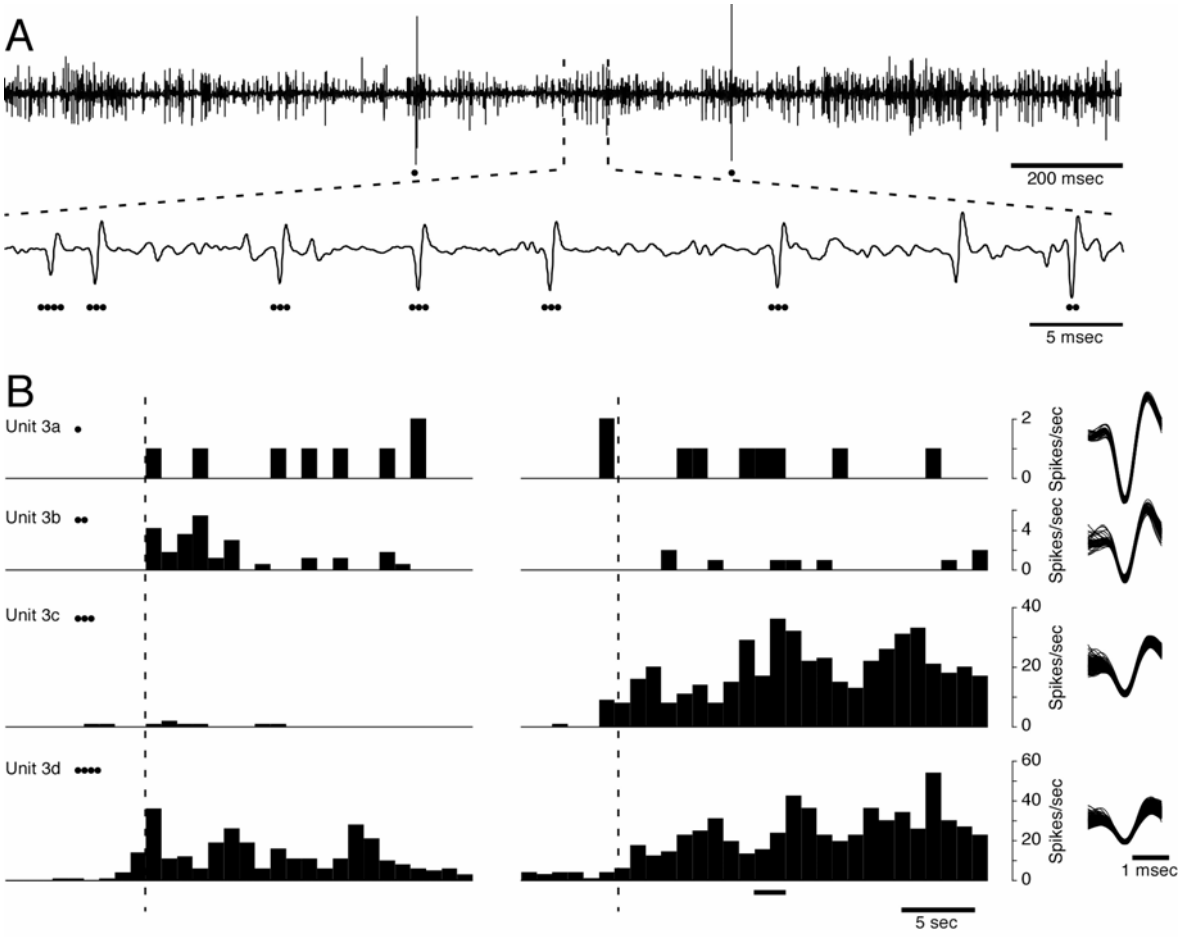
Circumesophageal
commissure

Figure 1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200 msec
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Spikes/sec
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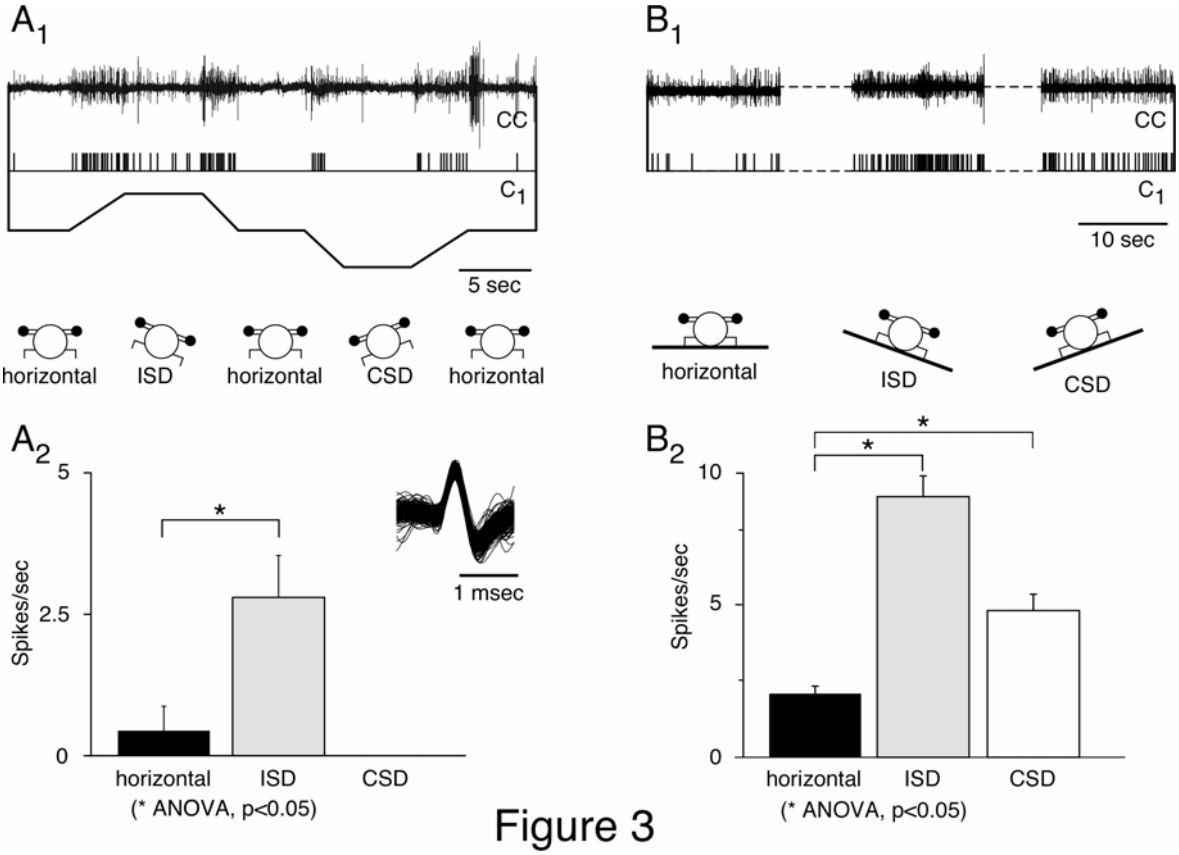
Figure 2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A2
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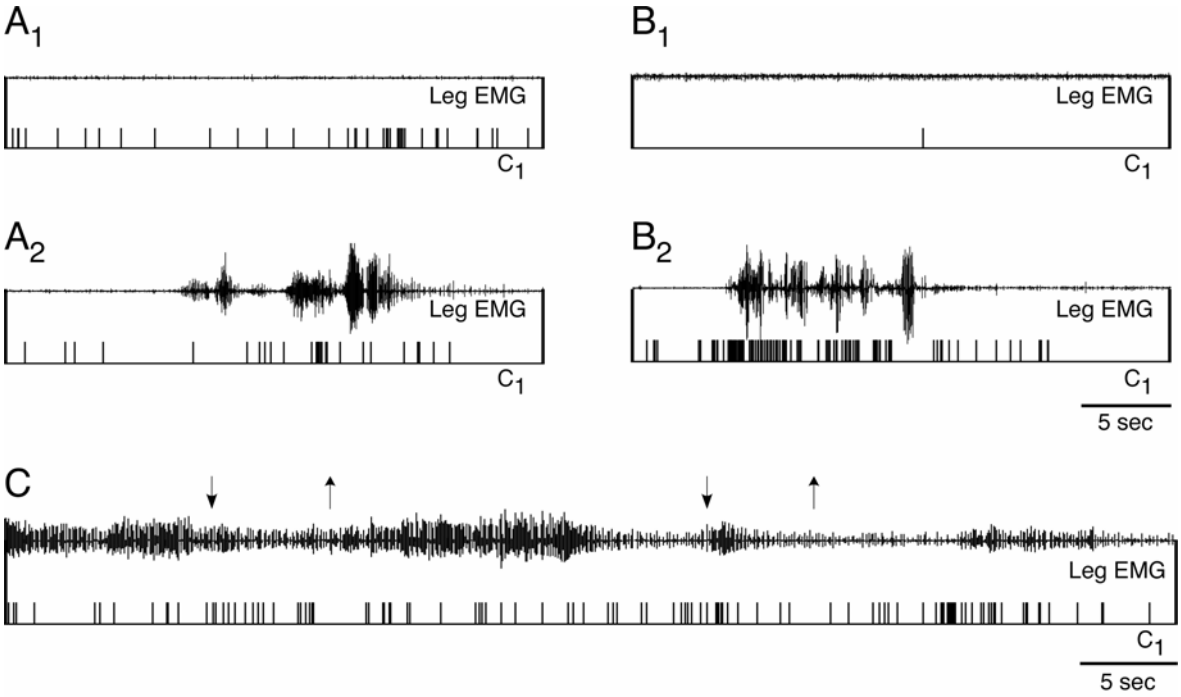
(* ANOVA, p<0.05)

Figure 3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C1
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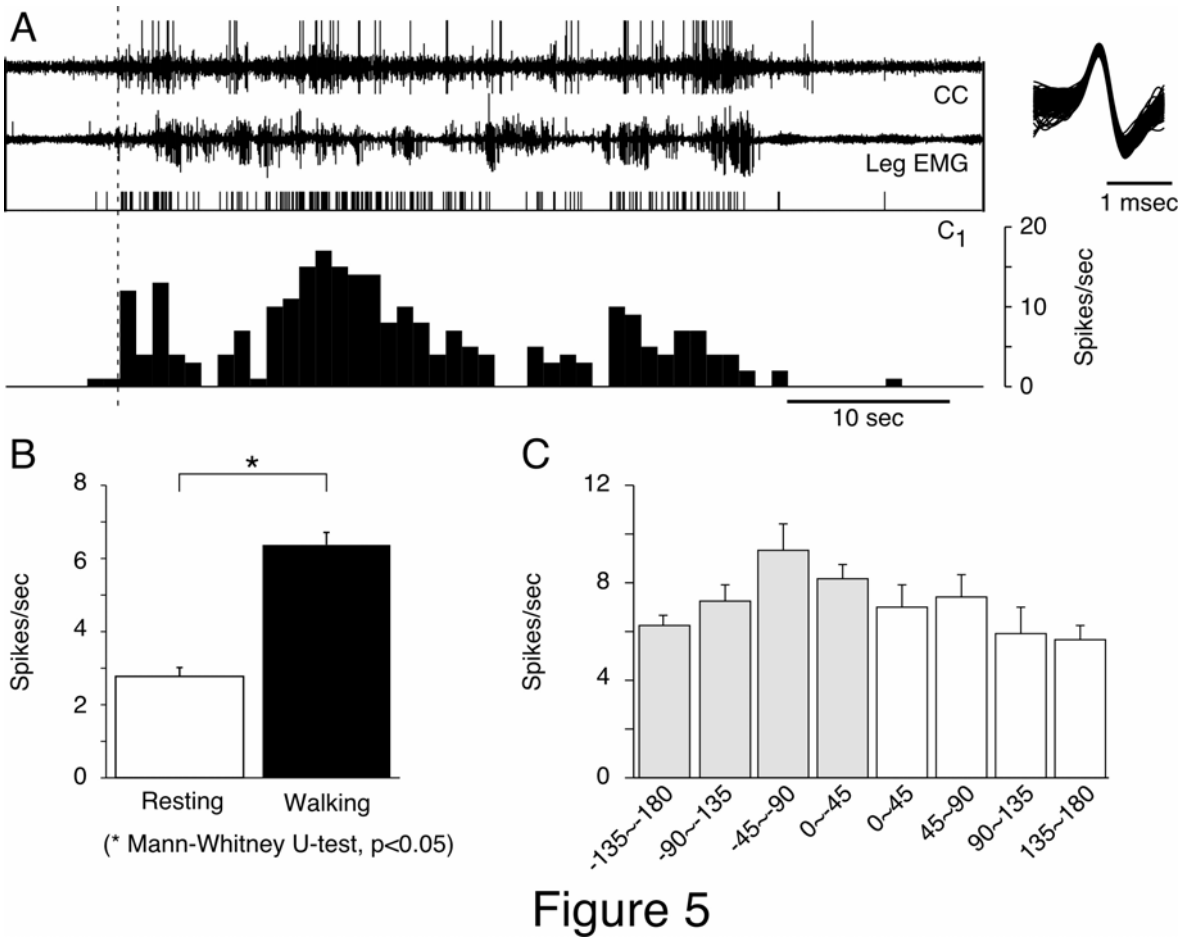
Figure 4


A
CC
Leg EMG
1 msec
C1
20
10
0
Spikes/sec
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B
*
8
6
4
2
0
Spikes/sec
Resting
Walking
(* Mann-Whitney U-test, p<0.05)
C
12
8
4
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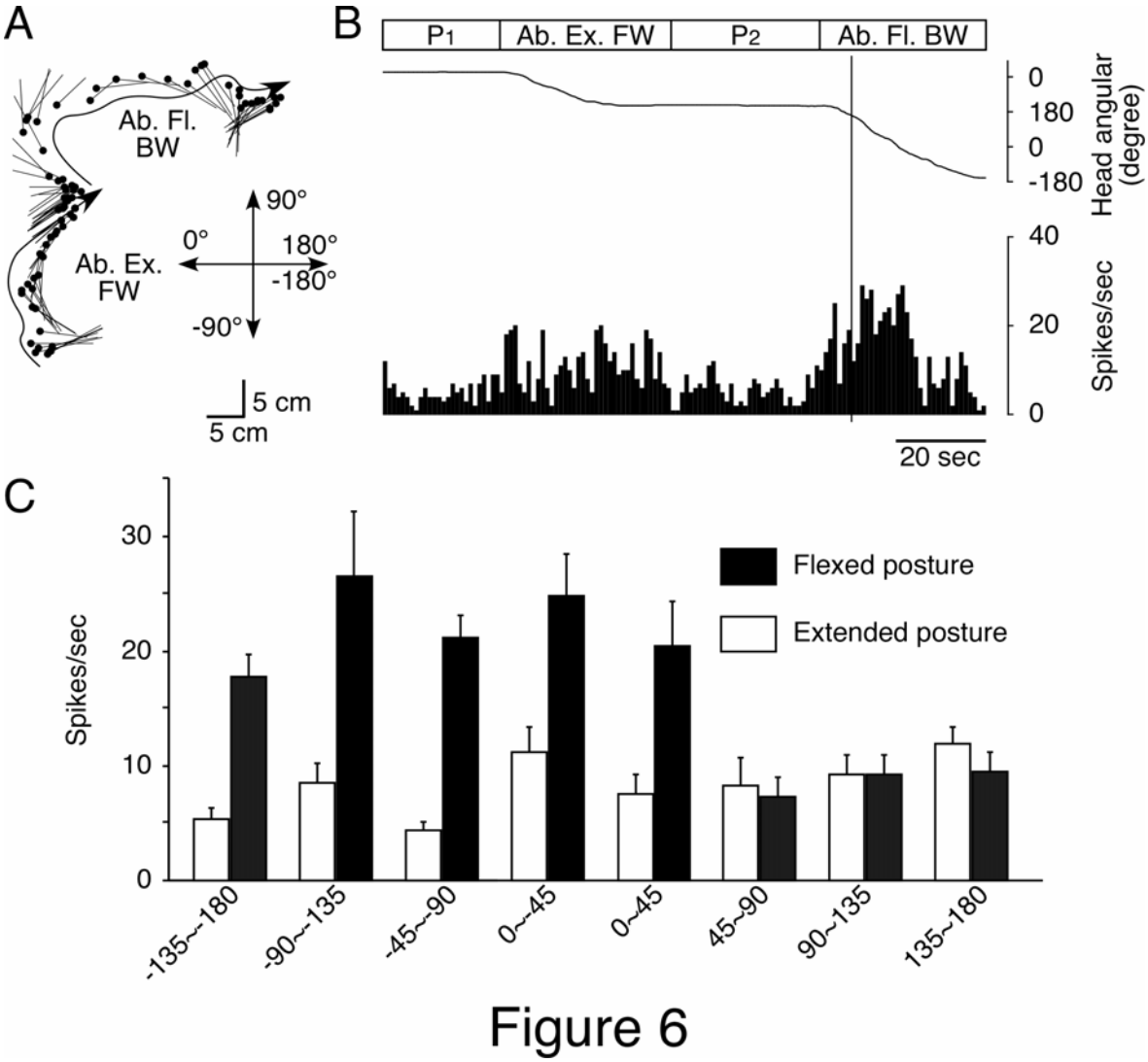
Spikes/sec
-135~-180
-90~-135
-45~-90
0~-45
0~45
45~90
90~135
135~180

Figure 5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-180°
-90°
5 cm
5 cm
B
P1
Ab. Ex. FW
P2
Ab. Fl. BW
0
180
0
-180
Head angular
(degree)
40
20
0
Spikes/sec
20 sec
C
Spikes/sec
30
20
10
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Flexed posture
Extended posture
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-90~-135
-45~-90
0~45
0~45
45~90
90~135
135~180

Figure 6

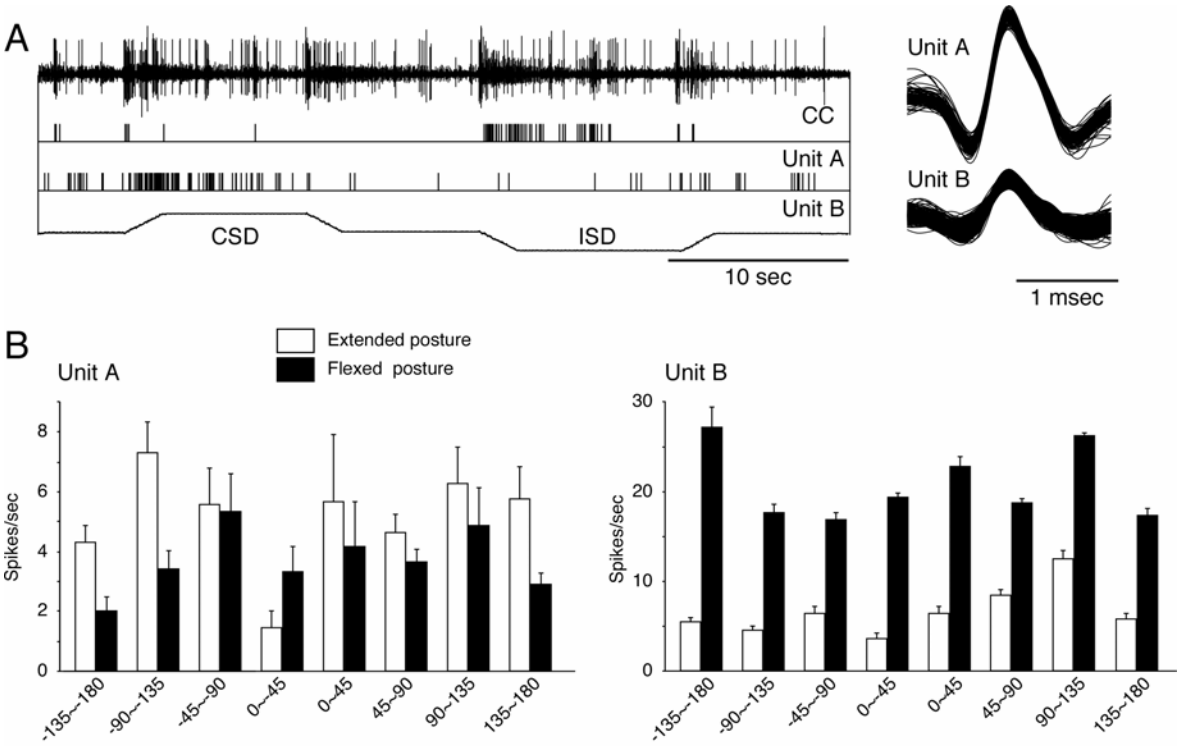
A
CC
Unit A
Unit B
CSD
ISD
10 sec
Unit A
Unit B
1 msec
B
Extended posture
Flexed posture
Unit A
Unit B
Spikes/sec
Spikes/sec
-135~-180
-90~-135
-45~-90
0~-45
0~45
45~90
90~135
135~180

Figure 7

Unit No	BodyTilting	Resting		Walking	
		Extended/Flexed	Resting/Walking	Forward/Backward	Extended/Flexed
1a	no response	=	<<	<	=
1b	no response	=	<<	<<	<<
1c	no response	=	<<	=	>>
1d*	ipsilateral side down/ head up	=	<<	<<	<<
1e	no response	=	<	<<	<<
1f	no response	>>	<<	=	=
2a	no response	=	<<	>>	>>
2b	no response	=	<<	=	>>
2c*	ipsilateral side down/ head up	=	<<	<<	<<
2d	no response	=	<<	<<	<
2e	no response	<<	<<	<<	<
2f	no response	=	<<	<<	<<
3a	no response	<<	>>	=	>>
3b	no response	<<	>>	=	=
3c	no response	<<	<<	<<	<<
3d	no response	=	<<	<<	<<
4a**	ipsilateral side down	<<	<<	<<	<<
4b***	contralateral side down	<<	<<	<<	<<
4c	no response	=	<<	<<	<<
4d	no response	=	<<	<	<<
4e	no response	=	<	<<	<<

*C_1 neuron **Unit A ***Unit B

<<; $p<0.05$ <; $p<0.1$

Table 1